

IT IS CLAIMED:

1. A method of sequencing in parallel a plurality of polynucleotide sample fragments, the method
5 comprising:

(a) providing a plurality of sample polynucleotide fragments,

(b) from said sample fragments, forming a mixture of different length sequencing fragments that are comple-
10 mentary to at least two different sample fragments, wherein (1) each sequencing fragment terminates at a predefined end with a known base or bases, and (2) each sequencing fragment contains an identifier tag sequence which identifies the sample fragment to which the
15 sequencing fragment corresponds and optionally, the terminating base-type of the fragment, wherein said forming includes the steps of

- (1) inserting said sample polynucleotide fragments into a plurality of identical vectors, to form
20 a mixture of sequencing vectors,
- (2) isolating a plurality of unique-sequence clones from said sequencing vector mixture,
- (3) hybridizing to each unique-sequence clone, a tagged primer containing (i) an identifier tag
25 sequence, and (ii) a first primer sequence located on the 3'-side of the tag sequence, to form a primer-vector hybrid, where a different identifier tag sequence is used to identify each unique-sequence clone,
- 30 (4) performing one or more chain extension reactions on each hybrid to form different-length sequencing fragments each terminating with a known base or bases, and

(5) combining the different-length sequencing fragments generated from the hybrids, to form said sequencing fragment mixture,

(c) separating said sequencing fragments on the basis of fragment length under conditions effective to resolve fragments differing in length by a single base, to produce a plurality of resolved size-separated bands,

(d) collecting the size-separated bands in separate aliquots,

(e) amplifying the identifier tag sequences in each aliquot to form multiple copies of oligonucleotides complementary to the identifier tag sequences, and optionally, multiple copies of the identifier tag sequences also,

(f) contacting each amplified aliquot with an array of immobilized different-sequence tag probes, each tag probe (1) being capable of hybridizing specifically with one of said identifier tag sequences or a tag sequence complement thereof, and (2) having an addressable location in said array, where said contacting is conducted under conditions effective to provide specific hybridization of the identifier tag sequences, or tag sequence complements, with the corresponding immobilized tag probes, to form a hybridization pattern on said array,

(g) from the hybridization pattern formed, determining a nucleotide sequence for at least one sample fragment.

2. The method of claim 1, wherein said amplifying includes repeated cycles of binding and extending a second primer which is complementary to the first primer sequence in the sequencing fragments, to generate multiple copies of a sequence complementary to the identifier tag sequence.

3. The method of claim 1, wherein each tagged primer additionally includes a second primer sequence which is located on the 5'-side of the tag sequence in the tagged primer, and said amplifying includes repeated
5 cycles of binding and extending third and fourth primers which correspond to said first and second primer sequences, to amplify the identifier tag sequences and their complements.

10 4. The method of claim 1, wherein
step (b)(1) is performed on a plurality of separate, different-sequence tag-vectors, each different-sequence tag-vector having (i) a cloning site, (ii) located on the 3'-side of the cloning site, a first vector primer
15 sequence which contains a vector-identifier tag region which is unique for each different-sequence tag-vector, to form a plurality of libraries of different-sequence tag-vectors,

step (b)(2) includes isolating at least one clone
20 from each different-sequence tag-vector clone library, and

step (b)(3) includes mixing together a clone isolated from each said different-sequence tag-vector library before said hybridizing, to form a clone mixture,
25 and the tagged primers in said hybridizing step additionally contain sequences complementary to each different vector-tag identifier sequence.

5. The method of claim 4, wherein said different-
30 sequence tag-vectors additionally contain a second vector primer sequence that is the same for all said vectors, and which is located on the 3'-side of said first vector primer sequence in each said vector.

6. The method of claim 1, wherein sequencing fragments are labeled with a fluorescent label.

7. The method of claim 5, wherein said fluorescent label is attached to the 5'-end of a sequencing fragment.

8. The method of claim 5, wherein said fluorescent label is attached to the 3'-end of a sequencing fragment.

9. The method of claim 5, wherein a different fluorescent label is used to identify each different terminating base-type.

10. The method of claim 1, wherein sequencing fragments are labeled with a radioactive label.

11. The method of claim 1, wherein detectable labels are incorporated into the sequencing fragments during said amplifying.

12. The method of claim 1, wherein said separating is effected by capillary electrophoresis.

13. The method of claim 1, wherein said separating is effected by slab gel electrophoresis.

14. A polynucleotide mixture comprising a plurality of primer-tag-primer polynucleotides each comprising a first primer sequence, an identifier tag sequence linked to the 3'-side of the first primer sequence, and a second primer sequence linked to the 3'-side of the tag sequence,

wherein the first primer sequences are identical to each other, the identifier tag sequence in each primer-tag-primer polynucleotides differs from the tag sequence

in every other primer-tag-primer polynucleotide, and the second primer sequences are identical to each other.

15. A sequencing fragment mixture comprising
5 a plurality of different-sequence sequencing fragments derived from a plurality of different sample polynucleotide templates, each different-sequence sequencing fragment containing
- 10 (1) a template-complement region derived from a selected sample template fragment and having a pre-determined base-type located at the 3'-end of the associated fragment, and
 - (2) at the 5'-end of the fragment, a primer-tag-primer region containing (i) a first primer
15 sequence, (ii) an identifier tag sequence linked to the 3'-side of the first primer sequence, and (iii) a second primer sequence linked to the 3'-side of the tag sequence,
- wherein the first primer sequences in the sequencing
20 fragments are identical to each other, the second primer sequences in said sequencing fragments are identical to each other, and the identifier tag sequence in each primer-tag-primer region uniquely identifies the sample fragment from which the sequencing fragment was derived,
25 and the sequencing fragment's 3'-terminal base type.

16. A kit for sequencing a plurality of polynucleotide fragments, said kit comprising
a plurality of primer-tag-primer polynucleotides
30 each comprising a first primer sequence, an identifier tag sequence linked to the 3'-side of the first primer sequence, and a second primer sequence linked to the 3'-side of the tag sequence, wherein the first primer sequences are identical to each other, the tag sequence
35 in each primer-tag-primer polynucleotides differs from the tag sequence in every other primer-tag-primer

polynucleotide, and the second primer sequences are identical to each other, and

an array of immobilized different-sequence tag probes, each tag probe (1) being capable of hybridizing
5 specifically with one of said identifier tag sequences or a tag sequence complement, and (2) having an addressable location in said array.